VARIANTS OF ALTERNATIVE SPLICING

FIELD OF THE INVENTION

The present invention concerns novel nucleic acid sequences, vectors and host cells containing them, amino acid sequences encoded by said sequences, and antibodies reactive with said amino acid sequences, as well as pharmaceutical compositions comprising any of the above. The present invention further concerns methods for screening for candidate activator or deactivators utilizing said amino acid sequences.

BACKGROUND OF THE INVENTION

Alternative splicing (AS) is an important regulatory mechanism in higher eukaryotes (P.A. Sharp, *Cell* 77, 805-8152 (1994). It is thought to be one of the important mechanisms for differential expression related to tissue or development stage specificity. It is known to play a major role in numerous biological systems, including human antibody responses, sex determination in *Drosophila*, and (S. Stamm, M.Q. Zhang, T.G. Marr and D.M. Helfman, *Nucleic Acids Research* **22**, 1515-1526 (1994); B. Chabot, *Trends Genet.* **12**, 472-478 (1996); R.E. Breitbart, A. Andreadis, B. Nadal-Ginard, *Annual Rev. Biochem.*, **56**, 467-495 (1987); C.W. Smith, J.G. Patton, B. Nadal-Ginard, *Annu. Rev. Genet.*, **27**, 527-577 (1989).

Until recently it was commonly believed that alternative splicing existed in only a small fraction of genes (about 5%). A recent observation based on literature survey of known genes revises this estimate to as high as stating that at least 30% of human genes are alternatively spliced (M.S. Gelfand, I. Dubchak, I. Draluk and M. Zorn, *Nucleic Acids Research* **27**, 301-302 (1999). The

importance of the actual frequency of this phenomenon lies not only in the direct impact on the number of proteins created (100,000 human genes, for example, would be translated to a much higher number of proteins), but also in the diversity of functionality derived from the process.

Several mechanisms at different stages may be held responsible for the complexity of higher eukaryote which include: alternative splicing at the transcription level, RNA editing at the post-transcriptional level, and post-translational modifications are the ones characterized to date.

GLOSSARY

In the following description and claims use will be made, at times, with a variety of terms, and the meaning of such terms as they should be construed in accordance with the invention is as follows:

"Variant nucleic acid sequence" – the sequence shown in any one of the sequences denoted NV_1 to NV_48611, which are listed in the attached CD-ROM marked *"New_Variants October 2000"* (hereinafter *"CD-ROM"*) sequences having at least *90% identity* (see below) to said sequence and *fragments* (see below) of the above sequences of least 20 b.p. long. The sequences are divided in 43 files according to their functional groups as will be explained hereinbelow. For convenience sake NV_1 to NV_48611 will be denoted SEQ ID NO: 1 to SEQ ID NO:48611, respectively in the following description. These sequences are sequences coding for novel, naturally occurring, alternative splice variants of native and known genes. It should be emphasized that the novel variants of the present invention are naturally occurring sequences resulting from alternative splicing of genes and not merely truncated, mutated or fragmented forms of known sequences. Thus the alternative splice variants of the invention have physiological significance as regards where, in what tissues, when, at which developmental stage and under which conditions (such as diseases, etc.) their expression is modulated, i.e., ceased, increased, up-regulated or down-regulated.

"Variant product – also referred at times as the "variant protein" or ***"variant polypeptide"*** – is an amino acid sequence encoded by the variant nucleic acid sequence which is a naturally occurring mRNA sequence obtained as a result of alternative splicing. The amino acid sequence may be a peptide, a protein, as well as peptides or proteins having *chemically modified* amino acids (see below) such as a glycopeptide or glycoprotein. The term also includes *homologues* (see below) of said sequences in which one or more amino acids has been added, deleted, *substituted* (see below) or *chemically modified* (see below) as well as *fragments* (see below) of this sequence having at least 10 amino acids. More specifically, it concerns the amino acid sequences present in the above CD-ROM. Each amino acid sequences has the same NV_... number as the nucleic acid sequence from which it was coded. The directory name of the file is the functional family name. The nucleic sequences file name is (family-name)_for_patent; and the protein sequences file is (family-name)_pep_patent. They are also denoted NV_1 to NV-48611,each protein having the same NV_... number as the nucleic acid sequence from which it was coded.

"Nucleic acid sequence" – a sequence composed of DNA nucleotides, RNA nucleotides or a combination of both types and may include natural nucleotides, chemically modified nucleotides and synthetic nucleotides.

"Amino acid sequence" – a sequence composed of any one of the 20 naturally appearing amino acids, amino acids which have been *chemically modified* (see below), or composed of synthetic amino acids.

"Fragment of variant nucleic acid sequence" – novel short stretch of nucleic acid sequences of at least 20 b.p., which does not appear as a continuous stretch in the *original nucleic acid sequence* (see below). The fragment may be a sequence which was previously undescribed in the context of the published RNA and which affects the amino acid sequence encoded by the known gene. For

example, where the variant nucleic includes a sequence which was not included in the original sequence (a sequence which was an intron in the original sequence) the fragment includes that additional sequence. The fragment may also be a region which is not an intron, which was not present in the original sequence. Another example is when the variant lacks a non-terminal region which was present in the original sequence. The two stretches of nucleotides spanning this region (upstream and downstream of this region) are brought together by splicing in the variant, but are spaced from each by that region in the original sequence and are thus not continuous. A continuous stretch of nucleic acids comprising said two stretches of nucleotides, is not present in the original sequence and they are spaced at present in the variant and thus fall under the definition of fragment.

"Fragments of variant products" - novel amino acid sequences coded by the *"fragment of variant nucleic acid sequence"* defined above.

"Homologues of variants" – amino acid sequences of variants in which one or more amino acids has been added, deleted or replaced. The addition, deletion or replacement should be in regions or adjacent to regions where the variant differs from the *original sequence* (see below).

"Conservative substitution" - refers to the substitution of an amino acid in one class by an amino acid of the same class, where a class is defined by common physicochemical amino acid side chain properties and high substitution frequencies in homologous proteins found in nature, as determined, for example, by a standard Dayhoff frequency exchange matrix or BLOSUM matrix. [Six general classes of amino acid side chains have been categorized and include: Class I (Cys); Class II (Ser, Thr, Pro, Ala, Gly); Class III (Asn, Asp, Gln, Glu); Class IV (His, Arg, Lys); Class V (Ile, Leu, Val, Met); and Class VI (Phe, Tyr,

Trp). For example, substitution of an Asp for another class III residue such as Asn, Gln, or Glu, is a conservative substitution.

"Non-conservative substitution" - refers to the substitution of an amino acid in one class with an amino acid from another class; for example, substitution of an Ala, a class II residue, with a class III residue such as Asp, Asn, Glu, or Gln.

"Chemically modified" - when referring to the product of the invention, means a product (protein) where at least one of its amino acid resides is modified either by natural processes, such as processing or other post-translational modifications, or by chemical modification techniques which are well known in the art. Among the numerous known modifications typical, but not exclusive examples include: acetylation, acylation, amidation, ADP-ribosylation, glycosylation, GPI anchor formation, covalent attachment of a lipid or lipid derivative, methylation, myristlyation, pegylation, prenylation, phosphorylation, ubiqutination, or any similar process.

"Biologically active" - refers to the variant product having some sort of biological activity, for example, some physiologically measurable effect on target cells, molecules or tissues.

"Immunologically active" defines the capability of a natural, recombinant or synthetic varient product, or any fragment thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies. Thus, for example, an immunologically active fragment of variant product denotes a fragment which retains some or all of the immunological properties of the variant product, e.g can bind specific anti-variant product antibodies or which can elicit an immune response which will generate such antibodies or cause proliferation of specific immune cells which produce variant.

"Optimal alignment" - is defined as an alignment giving the highest percent identity score. Such alignment can be performed using a variety of commercially available sequence analysis programs, such as the local alignment program LALIGN using a ktup of 1, default parameters and the default PAM. A preferred alignment is the one performed using the CLUSTAL-W program from MacVector (TM), operated with an open gap penalty of 10.0, an extended gap penalty of 0.1, and a BLOSUM similarity matrix. If a gap needs to be inserted into a first sequence to optimally align it with a second sequence, the percent identity is calculated using only the residues that are paired with a corresponding amino acid residue (i.e., the calculation does not consider residues in the second sequences that are in the *"gap"* of the first sequence). In case of alignments of known gene sequences with that of the new variant, the optimal alignment invariably included aligning the identical parts of both sequences together, then keeping apart and unaligned the sections of the sequences that differ one from the other.

"Having at least 90% identity" - with respect to two amino acid or nucleic acid sequence sequences, refers to the percentage of residues that are identical in the two sequences when the sequences are optimally aligned. Thus, 90% amino acid sequence identity means that 90% of the amino acids in two or more optimally aligned polypeptide sequences are identical, however this definition explicitly excludes sequences which are 100% identical with the original sequence from which the variant of the invention was varied.

"Isolated nucleic acid molecule having an variant nucleic acid sequence" - is a nucleic acid molecule that includes the coding variant nucleic acid sequence. Said isolated nucleic acid molecule may include the variant nucleic acid sequence as an independent insert; may include the variant nucleic acid sequence fused to an additional coding sequences, encoding together a fusion protein in which the variant coding sequence is the dominant coding sequence (for

example, the additional coding sequence may code for a signal peptide); the variant nucleic acid sequence may be in combination with non-coding sequences, e.g., introns or control elements, such as promoter and terminator elements or 5' and/or 3' untranslated regions, effective for expression of the coding sequence in a suitable host; or may be a vector in which the variant protein coding sequence is a heterologous.

"Expression vector" - refers to vectors that have the ability to incorporate and express heterologous DNA fragments in a foreign cell. Many prokaryotic and eukaryotic expression vectors are known and/or commercially available. Selection of appropriate expression vectors is within the knowledge of those having skill in the art.

"Deletion" - is a change in either nucleotide or amino acid sequence in which one or more nucleotides or amino acid residues, respectively, are absent.

"Insertion" or "addition" - is that change in a nucleotide or amino acid sequence which has resulted in the addition of one or more nucleotides or amino acid residues, respectively, as compared to the naturally occurring sequence.

"Substitution" - replacement of one or more nucleotides or amino acids by different nucleotides or amino acids, respectively. As regards amino acid sequences the substitution may be conservative or non- conservative.

"Antibody" – refers to IgG, IgM, IgD, IgA, and IgG antibody. The definition includes polyclonal antibodies or monoclonal antibodies. This term refers to whole antibodies or fragments of the antibodies comprising the antigen-binding domain of the anti-variant product antibodies, e.g. antibodies without the Fc portion, single chain antibodies, fragments consisting of essentially only the variable, antigen-binding domain of the antibody, etc.

"Treating a disease" - refers to administering a therapeutic substance effective to ameliorate symptoms associated with a disease, to lessen the severity or cure the disease, or to prevent the disease from occurring.

"***Detection***" – refers to a method of detection of a disease, disorder, pathological or normal condition. This term may refer to detection of a predisposition to a disease as well as for establishing the prognosis of the patient by determining the severity of the disease.

"Probe" – the variant nucleic acid sequence, or a sequence complementary therewith, when used to detect presence of other similar sequences in a sample. The detection is carried out by identification of hybridization complexes between the probe and the assayed sequence. The probe may be attached to a solid support or to a detectable label.

"Original sequence" – the amino acid or nucleic acid sequence from which the variant of the invention have been varied as a result of alternative slicing.

"Data carrier" – a medium for holding informational data which is in a computer readable form. It may be a magnetic or non-magnetic data carrier.

SUMMARY OF THE INVENTION

The present invention is based on the finding of novel, naturally occurring splice variants, which are naturally occurring sequences obtained by alternative splicing of known genes. The novel splice variants of the invention are not merely truncated forms, fragments or mutations of known genes, but rather novel sequences which naturally occur within the body of individuals.

Each novel splice variant is a result of alternative splicing of an original sequence. One original sequence may have one or more splice variant sequences

derived therefrom by alternative splicing. The original sequence and hence the variants have been divided to 43 functional groups according to their biological activity as will be explained below.

The nucleic acid sequence is present in one of sequences denoted NV_1 (hereinafter *"SEQ ID NO:1"*) to NV_48611 (hereinafter *"SEQ ID NO: 48611"*) which are present in an attached CD-ROM marked *"New Variants October 2000"* listed in a group of 43 computer files: the nucleic acid sequences are listed under (functional group name)_ for_patent. The amino acid sequences are listed under (functional group name)_pep_patent. This CD-ROM forms an integral part of this disclosure, and will be denoted hereinafter simply as *"CD-ROM"*.

The term *"alternative splicing"* in the context of the present invention and claims refers to: intron inclusion, exon exclusion, addition or deletion of terminal sequences in the variant as compared to the original sequences, as well as to the possibility of *"intron retention"*. Intron retention is an intermediate stage in the processing of RNA transcripts, where prior to production of fully processed mRNA the intron (naturally spliced in the original sequence) is retained in the variant. These intermediately processed RNAs may have physiological significance and are also within the scope of the invention.

The novel variant products of the invention may have the same physiological activity as the original peptide from which they are varied (although perhaps at a different level); may have an opposite physiological activity from the activity featured by the original peptide from which they are varied; may have a completely different, unrelated activity to the activity of the original from which they are varied; or alternatively may have no activity at all and this may lead to various diseases or pathological conditions. Both in the case where the variant has the same activity as well as an opposite activity as the original sequence, it may differ from the original sequence in various properties not directly connected to its biological activity such as in its stability, its clearance rate, tissue and cellular localization, its temporal pattern of expression, mechanisms for its up or down regulations, responses to agonists or antagonists, etc.

The novel variants may serve for detection purposes, i.e. their presence or level may be indicative of a disease, disorder, pathological or normal condition or alternatively the ratio between the level variants and the level original peptide from which they were varied, or the ratio to other variants (derived from the same original sequence) may be indicative to a disease, disorder, pathological or normal condition.

For example, for detectional purposes, it is possible to establish differential expression of various variants in various tissues. A certain variant may be expressed mainly in one tissue, while the original sequence from which it has been varied, or another variant derived from the same sequence, may be expressed mainly in another tissue. Understanding of the distribution of the variants in various tissues may be helpful in basic research, for understanding the physiological function of the genes as well as may help in targeting pharmaceuticals or developing pharmaceuticals.

The study of the variants may also be helpful to distinguish various stages in the life cycles of cells which may also be helpful for development of pharmaceuticals for various pathological conditions in which cell cycles is non-normal, for example cancer.

Thus the detection may by determination of the presence or the level of expression of the variant within a specific cell population, comprising determining said presence or level and comparing it between various cell types in a tissue, between different tissues and between individuals.

Thus the present invention provides by its first aspect, a novel isolated nucleic acid molecule comprising or consisting of any one of the coding sequence SEQ ID NO: 1 to SEQ ID NO: 48611, fragments of said coding sequence having at least 20 nucleic acids (provided that said fragments are continuous stretches of nucleotides not present in the original sequence from which the variant was varied), or a molecule comprising a sequence having at least 90%, identity to SEQ ID NO: 1 to SEQ ID NO:48611, provided that the molecule is not completely identical to the original sequence from which the variant was varied.

The present invention further provides a protein or polypeptide comprising or consisting of an amino acid sequence encoded by any of the above nucleic acid sequences, termed herein *"variant product"*, fragments of the above amino acid sequence having a length of at least 10 amino acids coded by the above fragments of the nucleic acid sequences, as well as homologues of the above amino acid sequences in which one or more of the amino acid residues has been substituted (by conservative or non-conservative substitution) added, deleted, or chemically modified. More specifically, the amino acid sequences are those present in the attached CD-ROM wherein each amino acid sequence has the same NV_... numbers as the nucleic acid sequence which codes for it.

The deletions, insertions and modifications should be in regions, or adjacent to regions, wherein the variant differs from the original sequence.

For example, where the variant is different from the original sequence by addition of a short stretch of 10 amino acids, in the terminal or non- terminal portion of the peptide, the invention also concerns homologues of that variant where the additional short stretch is altered for example, it includes only 8 additional amino acids, includes 13 additional amino acids, or it includes 10 additional amino acids, however some of them being conservative or non-conservative substitutes of the original additional 10 amino acids of the novel variants. In all cases the changes in the homolog, as compared to the original sequence, are in the same regions where the variant differs from the original sequence, or in regions adjacent to said region.

Another example is where the variant lacks a non-terminal region (for example of 20 amino acids) which is present in the original sequence (due for example to exon exclusion). The homologues may lack in the same region only 17 amino acids or 23 amino acids. Again the deletion is in the same region where the variant lacks a sequence as compared to the original sequence, or in a region adjacent thereto.

It should be appreciated that once a man versed in the art's attention is directed to the importance of a specific region, due to the fact that this region differs

in the variant as compared to the original sequence, there is no problem in derivating said specific region by addition to it, deleting from it, or substituting some amino acids in it. Thus homologues of variants which are derivated from the variant by changes (deletion, addition, substitution) only in said region as well as in regions adjacent to it are also a part of the present invention. Generally, if the variant is distinguished from the original sequence by some sort of physiological activity, then the homolog is distinguished from the original sequence in essentially the same manner.

The present invention further provides nucleic acid molecule comprising or consisting of a sequence which encodes the above amino acid sequences, (including the fragments and homologues of the amino acid sequences). Due to the degenerative nature of the genetic code, a plurality of alternative nucleic acid sequences, beyond those depicted in any one of SEQ ID NO:1 to SEQ ID NO:48611, can code for the amino acid sequence of the invention. Those alternative nucleic acid sequences which code for the same amino acid sequences coded by the sequence SEQ ID NO:1 to SEQ ID NO:48611 are also an aspect of the of the present invention.

The present invention further provides expression vectors and cloning vectors comprising any of the above nucleic acid sequences, as well as host cells transfected by said vectors.

The present invention still further provides pharmaceutical compositions comprising, as an active ingredient, said nucleic acid molecules, said expression vectors, or said protein or polypeptide.

These pharmaceutical compositions are suitable for the treatment of diseases and pathological conditions, which can be ameliorated, cured or prevented by raising the level of any one of the variant products of the invention.

By a second aspect, the present invention provides a nucleic acid molecule comprising or consisting of a non-coding sequence which is complementary to that of any one of SEQ ID NO:1 to SEQ ID NO:48611, or complementary to a sequence having at least 90% identity to said sequence (with the proviso added above) or a

fragment of said two sequences (according to the above definition of fragment). The complementary sequence may be a DNA sequence which hybridizes with any one of SEQ of ID NO:1 to SEQ ID NO:48611 or hybridizes to a portion of that sequence having a length sufficient to inhibit the transcription of the complementary sequence. The complementary sequence may be a DNA sequence which can be transcribed into an mRNA being an antisense to the mRNA transcribed from any one of SEQ ID NO:1 to SEQ ID NO:48611 or into an mRNA which is an antisense to a fragment of the mRNA transcribed from any one of SEQ ID NO:1 to SEQ ID NO:48611 which has a length sufficient to hybridize with the mRNA transcribed from SEQ ID NO:1 to SEQ ID NO:48611, so as to inhibit its translation. The complementary sequence may also be the mRNA or the fragment of the mRNA itself.

The nucleic acids of the second aspect of the invention may be used for therapeutic or diagnostic applications for example as probes used for the detection of the variants of the invention.

The presence of the variant transcript or the level of the variant transcript (identified either by any one of sequences 1 to 48611 or by a sequence complementary thereto) may be indicative of a multitude of diseases, disorders and various pathological as well as normal conditions. In addition, the ratio of the level of the transcripts of the variants of the invention may also be compared to that of the transcripts of the original sequences from which they were varied, or to the level of transcript of other variants, and said ratio may be indicative to a multitude of diseases, disorders and various pathological and normal conditions.

The present invention also provides expression vectors comprising any one of the above defined complementary nucleic acid sequences and host cells transfected with said nucleic acid sequences or vectors, being complementary to those specified in the first aspect of the invention.

The invention also provides anti-variant product antibodies, namely antibodies directed against the variant product which specifically bind to said variant product. Said antibodies are useful both for diagnostic and therapeutic

purposes. For example said antibodies may be as an active ingredient in a pharmaceutical composition as will be explained below.

By another alternative, the invention concerns antibodies termed *"distinguishing antibodies"* which are directed solely to the amino acid sequences which distinguishes the variant from the original amino acid sequence from which it has been varied by alternative splicing. For example, where the variant contains 15 additional amino acids as compared to the original sequence (due to intron inclusion) the antibodies may be directed against these additional amino acids (present in the variant and not present in the original sequence). Another example is where the variant lacks 20 amino acids as compared to the original sequence from which it is varied (for example due to exon exclusion). The distinguishing antibodies in that case may be directed only against these 20 amino acids which are present in the original sequence and absent from the variant sequence.

The antibodies and the distinguishing antibodies may be used for detection purposes, i.e. to detect individuals, tissue, conditions (both pathological or physiological) wherein the variant sequence or original sequence are evident or abundant. The antibodies may also be used to distinguish conditions where the level, or ratio of the variant to original sequence is altered.

The antibodies and the distinguishing antibodies may also be used for therapeutical purposes, i.e., to neutralize only the variant product or only the product of the original sequence, as the case may be, without neutralizing the other.

The present invention also provides pharmaceutical compositions comprising, as an active ingredient, the nucleic acid molecules which comprise or consist of said complementary sequences, or of a vector comprising said complementary sequences. The pharmaceutical composition thus provides pharmaceutical compositions comprising, as an active ingredient, said anti-variant product antibodies.

The pharmaceutical compositions comprising said anti-variant product antibodies or the nucleic acid molecule comprising said complementary sequence, are suitable for the treatment of diseases and pathological conditions where a

therapeutically beneficial effect may be achieved by neutralizing the variant (either at the transcript or product level) or decreasing the amount of the variant product or blocking its binding to its target, for example, by the neutralizing effect of the antibodies, or by the decrease of the effect of the antisense mRNA in decreasing expression level of the variant product.

The variant products of the invention may also be used for screening of pharmaceuticals which interact only with the variant and not with the original sequence, or *vice versa*, thereby choosing or tailoring pharmaceuticals having better specificity either to tissues, specific conditions or better specificity to proteins expressed by a specific individual.

According to the third aspect of the invention the present invention provides methods for detecting the level of the transcript (mRNA) of said variant product in a body fluid sample, or in a specific tissue sample, for example by use of probes comprising or consisting of said coding sequences; as well as methods for detecting levels of expression of said product in tissue, e.g. by the use of antibodies capable of specifically reacting with the variant products of the invention. Detection of the level of the expression of the variant of the invention in particular as compared to that of the original sequence from which it was varied or compared to other variant sequences all varied from the same original sequence may be indicative of a plurality of physiological or pathological conditions.

The method, according to this latter aspect, for detection of a nucleic acid sequence which encodes the variant product in a biological sample, comprises the steps of:

(a) providing a probe comprising at least one of the nucleic acid sequences defined above;

(b) contacting the biological sample with said probe under conditions allowing hybridization of nucleic acid sequences thereby enabling formation of hybridization complexes;

(c) detecting hybridization complexes, wherein the presence of the complex indicates the presence of nucleic acid sequence encoding the variant product in the biological sample.

The method as described above is qualitative, i.e. indicates whether the transcript is present in or absent from the sample. The method can also be quantitative, by determining the level of hybridization complexes and then calibrating said levels to determining levels of transcripts of the desired variant in the sample.

Both qualitative and quantitative determination methods can be used for diagnostic, prognostic and therapy planning purposes.

By a preferred embodiment the probe is part of a nucleic acid chip used for detection purposes, i.e. the probe is a part of an array of probes each present in a known location on a solid support.

The nucleic acid sequence used in the above method may be a DNA sequence an RNA sequence, etc; it may be a coding or a sequence or a sequence complementary thereto (for respective detection of RNA transcripts or coding-DNA sequences). By quantization of the level of hybridization complexes and calibrating the quantified results it is possible also to detect the level of the transcript in the sample. If desired, the detected level may be compared to that of the original sequence or compared to that of other splice variants, for example, those obtained from the same original sequence by alternative splicing.

Methods for detecting mutations in the region coding for the variant product are also provided, which may be methods carried-out in a binary fashion, namely merely detecting whether there is any mismatches between the normal variant nucleic acid sequence of the invention and the one present in the sample, or carried-out by specifically detecting the nature and location of the mutation.

The present invention also concerns a method for detecting variant product in a biological sample, comprising the steps of:

(a) contacting with said biological sample the antibody of the invention, thereby forming an antibody-antigen complex; and

(b) detecting said antibody-antigen complex

wherein the presence of said antibody-antigen complex correlates with the presence of variant product in said biological sample.

As indicated above, the method can be quantitized to determine the level or the amount of the variant in the sample, alone or in comparison to the level of the original amino acid sequence from which it was varied, and qualitative and quantitative results may be used for diagnostic, prognostic and therapy planning purposes.

By yet another aspect the invention also provides a method for identifying candidate compounds capable of binding to the variant product and modulating its activity (being either activators or deactivators). The method includes:

(i) providing a protein or polypeptide comprising an amino acid sequence substantially as coded by any one of SEQ ID NO:1 to 48611, or a fragment of such a sequence;

(ii) contacting a candidate compound with said amino acid sequence;

(iii) measuring the physiological effect of said candidate compound on the activity of the amino acid sequences and selecting those compounds which show a significant effect on said physiological activity.

The present invention also concerns compounds identified by the above methods described above, which compound may either be an activator of the variant product or a deactivator thereof.

As indicated above, the novel variants of the invention fall under 43 functional groups.

These groups have been defined by the activity of the original sequences from which the variants have been varied. The name of the group, its function, the number of the original sequences (genes) falling under that group, the number of splice variants falling under that group and the SEQ ID NOS. of the variants are given in Table 1 below.

FUNCTIONAL GROUP NAME	Total of original sequences	Total of New Variants	# of New Variants	Description of the proteins
ADAPTOR_BINDING	442	5525	1-5525	Proteins that are associated to other cell components, either by binding, interacting, or associating to them. This interaction is necessary for the protein's activity and/or structure.
ADHESION	72	1054	5526-6579	Proteins that serve as adhesion molecules between adjoining cells
APOLIPOPROTEINS	9	202	6580-6781	Proteins that are part of the lipoprotein particle and act as a recognition signal for the cellular binding and internalization of these particles.
APOPTOSIS	43	645	6782-7426	Proteins and enzymes that are involved in the apoptosis pathway, either by inducing or inhibiting it.
CANCER	224	2659	7427-10085	Proteins that are involved in cancer; oncogenes, DNA repair proteins, tumor markers and antigens, tumor suppressors, and cellular second messengers that participate in cancer.
CARBOXYLASE	17	301	10086-10386	Enzymes that add or remove CO_2 groups
CD	38	376	10387-10762	Cell surface antigens
CELL_CYCLE	64	677	10763-11439	Proteins and enzymes involved in controlling the cell cycle pathway, cellular growth, cell division, and cellular progression.
COAGULATION	8	24	11440-11463	Proteins involved in the blood coagulation pathway
CONVERTING_	7	109	11464-11572	Enzymes that convert one protein to another by specific cleavage of the precurser protein.
CYCLASE	8	27	11573-11599	Enzymes that convert triphosphate to cyclic monophosphate

DEGRADATION	69	906	11600-12505	Proteins and cellular enzymes involved in the degradation process of other proteins.
DEVELOPMENTAL	21	143	12506-12648	Proteins effecting development
DISEASE_RELATED	79	856	12649-13504	Proteins involved in a certain disease(s), either by contributing to it, or by acting as a marker for it
DOMAIN	81	655	13505-14159	proteins involved in protein-protein interactions
ESTERASE	30	209	14160-14368	Enzymes cleaving the ester bond between a chemical residue and a protein.
GROWTH_FACTORS	58	630	14369-14998	Growth factors, cytokines, interleukins, interferons, and lymphokines
HORMONES	51	492	14999-15490	Hormones, poietin proteins
HOUSEKEEPING	49	405	15491-15895	Homeobox, heat shock proteins and factors, chaperonin
HYDRO	99	1215	15896-17110	Enzymes that modify the hydroxyl group, such as hydrogenase, dehydrogenase, hydrolase, and hydroxylase
IMMUNO	113	1529	17111-18639	Proteins that are involved in the immune and complement systems such as; antigens and autoantigens, immunoglobulins, MHC and HLA proteins and their associated proteins.
INHIBITORS	87	1127	18640-19766	Inhibitors and suppressors of other proteins and enzymes.
KINASE	275	3077	19767-22843	Kinase
LIPASE	23	238	22844-23081	Lipase, phospholipase, and lysophospholipase
MATRIX	351	4224	23082-27305	All proteins compromising the cell matrix and cytoskeleton.
MODIFYING_ENZYMES	207	2103	27306-29408	Miscellaneous enzymes such as paraoxonase, GTPase, ATPase, anhydrase.
MUTASE	7	77	29409-29485	Mutases and superoxide dismutase.
NEURO	61	429	29486-29914	CNS related proteins and enzymes
OXIDASE	44	520	29915-30434	Oxidase and peroxidase

OXYGENASE	12	141	30435-30575	Oxygenase, mono- and dio oxygenase
PHOSPHATASE	88	884	30576-31459	Phosphatases and phosphorylases
PHOSPHOPROTEINS	22	294	31460-31753	Phosphoproteins and phospholipids
PROTEASE	113	1392	31754-33145	Proteases, peptidases, and proteinases.
RECEPTORS	205	1684	33146-34829	Receptors
REDUCTASE	60	721	34830-35550	Reductases
SECRETED_FACTORS	23	110	35551-35660	Secreted proteins
SIGNAL_TRANSDUCTION	51	490	35661-36150	Proteins that participate in signal transduction; such as G proteins,
SUBCELLULAR	53	975	36151-37125	Subcellular proteins such as ribosomal proteins
SYNTASE	88	1255	37126-38380	Syntase, sythases, synthetase
TRANSCRIPTIONAL RNA_DNA	502	6750	38381-45130	Nuclear proteins involved in RNA and DNA, such as transcription factors, RNA and DNA binding proteins, zinc fingers, helicase, isomerase, histones, nucleases,
TRANSFER	142	1423	45131-46553	Proteins involved in TRANSFER of functional groups
TRANSLATIONAL_ FACTORS	30	476	46554-47029	Proteins and enzymes involved in the translational process such as elongation and initiation factors
TRANSPORTER	171	1582	47030-48611	Proteins that mediate the transport of molecules and macromoleules, such as channels, exchangers, pumps.

The pharmaceutical compositions, whether comprising the nucleic acid sequences of the variants of the invention themselves (alone or in an expression vector), comprising complementary sequences thereto (alone or in an expression vector), comprising the amino acid (products), or alternatively, comprising antibodies to the above, are suitable for the treatment of a plurality of diseases, each

one in accordance with the activity of the functional group to which the new variant falls.

The detection of diseases utilizing a variant probe (comprising the variant sequence or a sequence complementary thereto) or alternatively comprising an amino acid sequence reactive with the variant product is also in accordance with the functional group to which the variants belong.

Thus, in the following, there shall be a brief summary of those conditions, and diseases in which the pharmaceutical composition can treat, i.e. cure, ameloriate or prevent, as well as those conditions which can be detected by variant probes of the present invention, or by antibodies reactive with the variant product of the invention.

Group 1 - Adaptor-binding - (SEQ ID NO:1–5525), the pharmaceutical compositions (comprising all aspects as indicated above) and the probes/antibodies may treat or detect, respectively, pathological conditions which are associated with non-normal protein activity or structure. Binding of the products of the variants of this family, or antibodies reactive therewith, can modulate a plurality of protein activities as well as change protein structure.

Group 2 - Adhesion - (SEQ ID NO:5526-6579), the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, conditions in which adhesion between adjoining cells is involved, typically conditions in which the adhesion is non-normal. Typical examples of such conditions are cancer conditions in which non-normal adhesion may cause and enhance the process of metastasis. Other examples of such conditions include conditions of non-normal growth and development of various tissues in which modulation adhesion among adjoining cells can improve the condition.

Group 3 - Apolipoproteins - (SEQ ID NO:6580-6781), the pharmaceutical compositions (including the variant sequence, the product, a sequence

complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, diseases which involve non-normal lipoprotein particles signaling, cellular binding and internalization, such as diseases which involve abnormally high or low levels of lipoprotein and cholesterol, as well as conditions involved in the formation or artherosclerosis.

Group 4 - Apoptosis - (SEQ ID NO:6782-7426), the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence and antibody may serve to treat, or detect, respectively, diseases which are involved in premature death of cells, such as degenerative diseases, for example neurodegenerative diseases or conditions associated with aging, or alternatively, diseases wherein apoptosis which should have taken place, does not take place. Example of such diseases are cancerous diseases.

Group 5 - Cancer diseases - (SEQ ID NO:7427-10085) the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibodies may serve to treat, or detect, respectively, cancer diseases as well as metastasis or prevent cancer diseases. The detection may also be for pre-disposition to a disease, as well as for determination of the stage of thedisease.

Group 6 - Carboxylases, (SEQ ID NO:10086-10386), the pharmaceutical compositions (including the variant sequence, the product, the sequence complementary to the variant sequence or an antibody to the product), and a probe variant sequence may serve to treat, or detect, respectively, these diseases which can be ameliorated or improved by regulation of enzymatic reactions which remove CO_2 groups from other moieties, notably be removal of CO_2 from protein.

Group 7 – CD's, (SEQ ID NO:10387-10762), the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or

antibody may serve to treat, or detect, respectively, diseases in which regulation of the recognition, or participation or bind of cell surface antigens to other moieties may improve the disease. These diseases include autoimmune diseases, various infectious diseases, cancer diseases which involve non cell surface antigens recognition and activity, etc.

Group 8 - Cell cycle, (SEQ ID NO:10763-11439), the pharmaceutical compositions (including the variant sequence, the product, the sequence complementary to the variant sequence or an antibody to the product), and a probe variant sequence may serve to treat, or detect, respectively, diseases which are manifested, or involved in non-normal cell cycle pathways, non-normal cellular growth division and progression. Typically these diseases are manifested either by degenerative diseases (low growth), or on the other hand by cancerous diseases (uncontrolled growth).

Group 9 - Coagulation, (SEQ ID NO:11440-11463), the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, diseases which are manifested by non-normal coagulation processes, which may include abnormal bleeding or excessive coagulation.

Group 10 - converting enzymes, (SEQ ID NO:11464-11572), the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, diseases which are manifested by non-normal conversion of one protein to the other due to lack or excessive cleavage of the specific precursor protein.

Group 11 - Cyclase, (SEQ ID NO:11573-11599), the pharmaceutical compositions (including the variant sequence, the product, the sequence complementary to the variant sequence or an antibody to the product), and a probe variant sequence may serve to treat, or detect, respectively, diseases which are manifested by non-normal (excessive, or lack of) conversion of trisphosphate to cyclic monophosphate, as

well as diseases in which internal-cell signaling, caused by the above conversion is non-normal.

Group 12 - Degradation, (SEQ ID NO:11600-12505) the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, diseases in which there is abnormal degradation of other proteins, which may cause non-normal accumulation of various proteinaceous products in cells, caused non-normal (prolonged or shortened) activity of proteins, etc.

Group 13 - Developmental, (SEQ ID NO:12506-12648), the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, diseases which are manifested by non-normal development, which may be non-normal development of the organism (genetic diseases involving non-normal development of a fetus), non-normal development of a tissue (a tissue which is not properly developed) as well as cancer diseases.

Group 14 - Disease-related, (SEQ ID NO:12649-13504), the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, a variety of different diseases.

Group 15 - Domain proteins, (SEQ ID NO:13505-14159), the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, diseases in which the protein-to-protein interactions are non-normal, due to excessive interaction, insufficient interaction or lack of proper interaction between proteins.

Group 16 - Esterase, (SEQ ID NO:14160-14368), the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, to diseases and pathological conditions which may be ameliorated by modulating the rate, or the ctivity of cleavage of ester bonds between any chemical residue and a protein.

Group 17 - Growth factors, (SEQ ID NO:14369-14998), the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, diseases which may be ameliorated by modulating the concentration or ativity or interaction binding, etc. of growth factors, cytokines, interleukins, interferon and lymphokines, typically such diseases such as autoimmune diseases, inflammation related disease, Graff vs. Host diseases, diseases caused by infectious agents, cancer diseases, as well as disease originating from improper concentration of growth factors causing non-normal (either excessive or too little of) growth of various tissues themselves, or causing untimely death of a desired cell population.

Group 18 - Hormones, (SEQ ID NO:14999-15490) the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, diseases which are endocrine in essence (cause or are a result of hormones), or may be ameliorated by raising, or decreasing the level of hormones and poietin protein.

Group 19 - Housekeeping, (SEQ ID NO:15491-15895) the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, conditions in which a beneficial effect is evident by regulating the expression of a protein necessary for survival.

Group 20 - Hydro, (SEQ ID NO:15896-17110) the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, diseases in which the activity connected with hydroxyl groups such as hydrogenation, dehydrogenation, hydrolation, and hydroxylation activity is non-normal (increased or decreased).

Group 21 - Immuno, (SEQ ID NO:17111-18639) the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, diseases involving the immunological system including inflammation, autoimmune diseases, infectious diseases, as well as cancerous processes.

Group 22 - Inhibitors, (SEQ ID NO:18640-19766) the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, diseases in which beneficial effect may be achieved by modulating the activity of inhibitors and suppressors of proteins and enzymes.

Group 23 - Kinase, (SEQ ID NO:19767-22843), the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, diseases which may be ameliorated by a modulating kinase activity, which is one of the main signaling pathways inside cell.

Group 24 - Lipase, (SEQ ID NO:22844-23081), the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence may serve to treat, or detect, respectively, diseases which involve non-normal metabolism activity or interactions of lipases.

Group 25 - Matrix, (SEQ ID NO:23082-27305), the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, diseases which are caused or due to abnormalities in cytoskelaton, including cancerous cells, and diseased cells including those which do not propagate, grow or function normally.

Group 26 - Modifying enzymes, (SEQ ID NO:27306-29408) the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, diseases which can be ameliorated by modulating the activity of various enzymes such as GTPases, ATPases, anhydrases and paraoxonases and various enzymes which are involved both in enzymatic processes inside cells as well as in cell signaling.

Group 27 - Mutase, (SEQ ID NO: 29409-29485) the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, diseases involving mutases and superoxidedismutases, including cancer diseases, and various other pathological processes connected with aging.

Group 28 - Neuro, (SEQ ID NO: 28486-29914) the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, diseases involving the central nervous system, including diseases involved in various types of dementia, neurodegenerative diseases, etc., diseases involving epilepsy, various psychiatric disorders, etc., cancer of neural origin.

Group 29 - Oxidase, (SEQ ID NO: 29915-30434) the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or

antibody may serve to treat, or detect, respectively, diseases caused by non-normal activity of improved oxidases and peroxidases.

Group 30 - Oxygenase, (SEQ ID NO:30435-30575) the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, diseases involving non-normal activity of oxygenases, mono- and dio-oxygenases.

Group 31 - Phosphatase, (SEQ ID NO:30576-31459) the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, diseases which can be ameliorated or cured by modulating the activity of phosphatases and phosphorylases.

Group 32 – Phosphoproteins, (SEQ ID NO:31460-31753) the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, diseases which involve phosphoproteins and phospholipids, i.e. diseases which are caused by an excess of, lack of or non-normal phosphoproteins or phospholipids.

Group 33 – Protease, (SEQ ID NO:31754-33145) the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, diseases which can be ameliorated by modulating the activity of proteases, peptidases and proteinases.

Group 34 - Receptors, (SEQ ID NO:33146-34829) the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, diseases involving various receptors present on various membranes in different

tissues of the body, including receptors to neurotransmitters, hormones and various other effectors and ligands.

Group 35 - Reductase, (SEQ ID NO: 34830-35550) the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, diseases involving the reductases enzymes.

Group 36 - Secreted-factors, (SEQ ID NO:35551-35660) the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, diseases which involve non-normal secretion of proteins which may be due to non-normal presence, absence or non-normal response to normal levels of secreted proteins including hormones, neurotransmitters, and various other proteins secreted by cells to the extracellular environment.

Group 37 - Signal-transduction, (SEQ ID NO:35661-36150) the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, diseases in which the signal-transduction, typically involving G-proteases is non-normal, either as a cause, or as a result of the disease.

Group 38 - Sub-cellular, (SEQ ID NO: 36151-37125) the pharmaceutical compositions (including the variant sequence, the product, the sequence complementary to the variant sequence or an antibody to the product), and a probe variant sequence may serve to treat, or detect, respectively, diseases involving non-normal sub-cellular proteins such as non-normal ribozymal protein.

Group 39 – Synthase, (SEQ ID NO: 37126-38380) the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the

probe variant sequence or antibody may serve to treat, or detect, respectively, diseases in which the synthase, synthethase activity should be modulated.

Group 40 – Transcriptional RNA-DNA, (SEQ ID NO:38381-45130) the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, diseases involving transcription factors such as: helicases, isomerases, histones and nucleases, for example diseases where there is non-normal replication or transcription of DNA and RNA respectively.

Group 41 – Transfer, (SEQ ID NO:45131-46553) the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, diseases in which the transfer of functional group to a modulated moiety is not normal so that a beneficial effect may be achieved by modulation of such transfer.

Group 42 – Translational-factors, (SEQ ID NO:46554-47029) the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, diseases in which the translation, elongation and initiation is non-normal leading to various pathological conditions.

Group 43 – Transporters, (SEQ ID NO:47030-48611) the pharmaceutical compositions (including the variant sequence, the product, a sequence complementary to the variant sequence or an antibody to the product), and the probe variant sequence or antibody may serve to treat, or detect, respectively, diseases in which the transport of molecules and macromolecules such as neurotransmitters, hormones, sugar etc. is non-normal leading to various pathologies.

The present invention further concerns any one of SEQ ID NO:1 to SEQ ID NO:48611 present on a data carrier. The invention further concerns the amino cid sequences present on a data carrier.

The present invention further concerns such a data carrier for use in an analysis of a nucleic acid sequence or amino acid sequence. For the purpose of the analysis said nucleic acid sequence is compared to a sequence of a plurality of nucleic acid sequences being substantially SEQ ID NO: 1 to SEQ ID NO.48611 of which are present on a data carrier or alternatively to the plurality of amino acid sequences present on the carrier. Thus, the data carrier of the invention may be used by others for analysis of nucleic acid sequences which they have, in order to determine whether the sequence they have is a sequence of splice variants of a known gene, obtained through alternative splicing.

This may be done by using a software data combination comprising a nucleotide search and comparison software and a data carrier comprising all of the variant sequences of the invention. When the combination is loaded into the computer it can execute a search where a nucleotide sequence entered by the user is compared to the plurality of sequences comprising said data.

The software used for search and comparison between nucleic acid sequences is in combination with the data of the invention, may be any software known in the art for finding homology, at a specified level between an entered nucleic acid sequence and a plurality of nucleic acid sequences present on a data base any person wishing to determine whether a nucleic acid sequence he has is a splice variant of one of the original sequence, may do so by determining whether it appears in one of the sequences of the invention.

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DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Example 1: Explanation of the CD-ROM

The CD-ROM contains basically three files. A first file termed (protein family name)_for_patent include all the nucleic acid sequences of the invention. The sequences are arranged according to the functional (family) group, so that there exists 43 files, each one is named in accordance with the functional group name, as depicted in Table 1, for example, the first group is named, *"Adaptor_for_patent"*;

This group includes all splice variants from SEQ ID NO:1 to SEQ ID NO:5525 (termed in the sequence as NV_1 to NV_5525).

The second file is named *"Adhesion_for_patent"*, and includes those sequences from SEQ ID NO:5526-6579, termed NV_5526 to NV_6579 etc.

The amino acid sequences come inside each directory named (protein family name)_pep_patent. Thus there are two different files: 1. protein family name_for_patent (containing nucleotides); and 2. (protein family name)_pep_patent (containing amino acids) and are preceded by the same NV_number.

Preceding the actual sequence of each novel variant of the invention is the name of the original sequence from which it is varied, as well as the GenBank accession number of the original gene.

Between the name of the original sequence and its accession number, is present in the number of the novel variant of the invention preceded by **NV_**. Thus SEQ ID NO:1 is marked as – NV_1.;SEQ ID NO:2 is marked as NV_2, etc.

Since many times several novel variant sequences originate from the same original sequence, all of these novel variants originating from the same origin will be preceded by the description of the same original sequence and its accession number repeated again and again.

The CD-ROM also includes "*Table_summary_new.doc*" (which is identical in fact to Table 1).

Another table (file) present on the CD-ROM is (IP_OctOO.mdb) (Table 2). This table contains the names of all new variants, arranged by their SEQ ID NO., beginning from NV_1 and ending in NV_48611. After each new ID No there is the "Old ID" which is the number of the sequence (NV_) as appeared in the priority document. For example, NV_4 in the priority document is NV_3 in the present case. After the variant indexes comes the description given in GeneBank of the original sequence from which it has been varied, and after it the accession number of the original sequence from which it has been varied. Where several novel variants are varied from the same original sequence, the description and accession number of several consecutive lines will be identical. This table "*IP_Oct OO.mdb* " (Table 2) can be used for both nucleotides and amino acids.

Table 3 termed: "*Clear_Patent 1.doc*", concerns the NV_(or SEQ ID) Nos. of the priority document versus those of the present application.

Example II: Variant nucleic acid sequence

The nucleic acid sequences of the invention include nucleic acid sequences which encode variant product and fragments and analogs thereof. The nucleic acid sequences may alternatively be sequences complementary to the above coding sequence, or to a region of said coding sequence. The length of the complementary sequence is sufficient to avoid the expression of the coding sequence. The nucleic acid sequences may be in the form of RNA or in the form of DNA, and include messenger RNA, synthetic RNA and DNA, cDNA, and genomic DNA. The DNA may be double-stranded or single-stranded, and if single-stranded may be the coding strand or the non-coding (anti-sense, complementary) strand. The nucleic acid sequences may also both include dNTPs, rNTPs as well as non naturally occurring sequences. The sequence may also be a part of a hybrid between an amino acid sequence and a nucleic acid sequence.

In a general embodiment, the nucleic acid sequence has at least 90%, identity or 45% with any one of the sequence identified as SEQ ID NO: 1 to SEQ ID NO: 48611 provided that this sequence is not completely identical with that of the original sequence.

The nucleic acid sequences may include the coding sequence by itself. By another alternative the coding region may be in combination with additional coding sequences, such as those coding for fusion protein or signal peptides, in combination with non-coding sequences, such as introns and control elements, promoter and terminator elements or 5' and/or 3' untranslated regions, effective for expression of the coding sequence in a suitable host, and/or in a vector or host environment in which the variant nucleic acid sequence is introduced as a heterologous sequence.

The nucleic acid sequences of the present invention may also have the product coding sequence fused in-frame to a marker sequence which allows for purification of the variant product. The marker sequence may be, for example, a hexahistidine tag to provide for purification of the mature polypeptide fused to the marker in the case of a bacterial host, or, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, I., *et al. Cell* **37**:767 (1984)).

Also included in the scope of the invention are fragments as defined above also referred to herein as oligonucleotides, typically having at least 20 bases, preferably 20-30 bases corresponding to a region of the coding-sequence nucleic acid sequence. The fragments may be used as probes, primers, and when complementary also as antisense agents, and the like, according to known methods.

As indicated above, the nucleic acid sequence may be substantially a depicted in any one of SEQ ID NO:1 to SEQ ID NO:48611 or fragments thereof or sequences having at least 90% identity to the above sequence as explained above. Alternatively, due to the degenerative nature of the genetic code, the

sequence may be a sequence coding for any one of the amino acid sequence coded by the sequence of SEQ ID NO:1 to SEQ ID NO:48611, or fragments or analogs of said amino acid sequence.

A. Preparation of nucleic acid sequences

The nucleic acid sequences may be obtained by screening cDNA libraries using oligonucleotide probes which can hybridize to or PCR-amplify nucleic acid sequences which encode the variant products disclosed above. cDNA libraries prepared from a variety of tissues are commercially available and procedures for screening and isolating cDNA clones are well-known to those of skill in the art. Such techniques are described in, for example, Sambrook *et al.* (1989) Molecular Cloning: A Laboratory Manual (2nd Edition), Cold Spring Harbor Press, Plainview, N.Y. and Ausubel FM et al. (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York, N.Y.

The nucleic acid sequences may be extended to obtain upstream and downstream sequences such as promoters, regulatory elements, and 5' and 3' untranslated regions (UTRs). Extension of the available transcript sequence may be performed by numerous methods known to those of skill in the art, such as PCR or primer extension (Sambrook *et al., supra*), or by the RACE method using, for example, the Marathon RACE kit (Clontech, Cat. # K1802-1).

Alternatively, the technique of *"restriction-site"* PCR (Gobinda *et al. PCR Methods Applic.* **2**:318-22, (1993)), which uses universal primers to retrieve flanking sequence adjacent a known locus, may be employed. First, genomic DNA is amplified in the presence of primer to a linker sequence and a primer specific to the known region. The amplified sequences are subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR can be used to amplify or extend sequences using divergent primers based on a known region (Triglia, T. *et al.*, *Nucleic Acids Res.* **16**:8186,

(1988)). The primers may be designed using OLIGO(R) 4.06 Primer Analysis Software (1992; National Biosciences Inc, Plymouth, Minn.), or another appropriate program, to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68-72°C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Capture PCR (Lagerstrom, M. *et al., PCR Methods Applic.* **1**:111-19, (1991)) is a method for PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA. Capture PCR also requires multiple restriction enzyme digestions and ligations to place an engineered double-stranded sequence into a flanking part of the DNA molecule before PCR.

Another method which may be used to retrieve flanking sequences is that of Parker, J.D., *et al., Nucleic Acids Res.*, **19**:3055-60, (1991)). Additionally, one can use PCR, nested primers and PromoterFinder™ libraries to *"walk in"* genomic DNA (PromoterFinder™; Clontech, Palo Alto, CA). This process avoids the need to screen libraries and is useful in finding intron/exon junctions. Preferred libraries for screening for full length cDNAs are ones that have been size-selected to include larger cDNAs. Also, random primed libraries are preferred in that they will contain more sequences which contain the 5' and upstream regions of genes.

A randomly primed library may be particularly useful if an oligo d(T) library does not yield a full-length cDNA. Genomic libraries are useful for extension into the 5' nontranslated regulatory region.

The nucleic acid sequences and oligonucleotides of the invention can also be prepared by solid-phase methods, according to known synthetic methods. Typically, fragments of up to about 100 bases are individually synthesized, then joined to form continuous sequences up to several hundred bases.

B. Use of variant nucleic acid sequence for the production of variant products

In accordance with the present invention, nucleic acid sequences specified above may be used as recombinant DNA molecules that direct the expression of variant products.

As will be understood by those of skill in the art, it may be advantageous to produce variant product-encoding nucleotide sequences possessing codons other than those which appear in any one of SEQ ID NO:1 to SEQ ID NO:48611 which are those which naturally occur in the human genome. Codons preferred by a particular prokaryotic or eukaryotic host (Murray, E. *et al. Nuc Acids Res.*, **17**:477-508, (1989)) can be selected, for example, to increase the rate of variant product expression or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life, than transcripts produced from naturally occurring sequence.

The nucleic acid sequences of the present invention can be engineered in order to alter a variant product coding sequence for a variety of reasons, including but not limited to, alterations which modify the cloning, processing and/or expression of the product. For example, alterations may be introduced using techniques which are well known in the art, e.g., site-directed mutagenesis, to insert new restriction sites, to alter glycosylation patterns, to change codon preference, etc.

The present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a nucleic acid sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. Appropriate cloning and

expression vectors for use with prokaryotic and eukaryotic hosts are also described in Sambrook, *et al., (supra)*.

The present invention also relates to host cells which are genetically engineered with vectors of the invention, and the production of the product of the invention by recombinant techniques. Host cells are genetically engineered (i.e., transduced, transformed or transfected) with the vectors of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the expression of the variant nucleic acid sequence. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to those skilled in the art.

The nucleic acid sequences of the present invention may be included in any one of a variety of expression vectors for expressing a product. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used as long as it is replicable and viable in the host. The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and related sub-cloning procedures are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate transcription control sequence (promoter) to direct mRNA synthesis. Examples of such promoters include: LTR or SV40 promoter, the *E.coli lac* or *trp* promoter, the phage lambda *PL* promoter, and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses.

The expression vector also contains a ribosome binding site for translation initiation, and a transcription terminator. The vector may also include appropriate sequences for amplifying expression. In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in *E.coli*.

The vector containing the appropriate DNA sequence as described above, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the protein. Examples of appropriate expression hosts include: bacterial cells, such as *E.coli*, *Streptomyces, Salmonella typhimurium*; fungal cells, such as yeast; insect cells such as *Drosophila* and *Spodoptera* Sf9; animal cells such as CHO, COS, HEK 293 or Bowes melanoma; adenoviruses; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein. The invention is not limited by the host cells employed.

In bacterial systems, a number of expression vectors may be selected depending upon the use intended for the variant product. For example, when large quantities of variant product are needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be desirable. Such vectors include, but are not limited to, multifunctional *E.coli* cloning and expression vectors such as *Bluescript*(R) (Stratagene), in which the variant polypeptide coding sequence may be ligated into the vector in-frame with sequences for the amino-terminal Met and the subsequent 7 residues of beta-galactosidase so that a hybrid protein is produced; *pIN* vectors (Van Heeke & Schuster *J. Biol. Chem.* **264**:5503-5509, (1989)); *pET* vectors (Novagen, Madison WI); and the like.

In the yeast *Saccharomyces cerevisiae* a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase and

PGH may be used. For reviews, see Ausubel *et al. (supra)* and Grant *et al.*, (*Methods in Enzymology* **153**:516-544, (1987)).

In cases where plant expression vectors are used, the expression of a sequence encoding variant product may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of *CaMV* (Brisson *et al., Nature* **310**:511-514. (1984)) may be used alone or in combination with the omega leader sequence from TMV (Takamatsu *et al., EMBO J.*, **6**:307-311, (1987)). Alternatively, plant promoters such as the small subunit of RUBISCO (Coruzzi *et al., EMBO J.* **3**:1671-1680, (1984); Broglie *et al., Science* **224**:838-843, (1984)); or heat shock promoters (Winter J and Sinibaldi R.M., *Results Probl. Cell Differ.,* **17**:85-105, (1991)) may be used. These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. For reviews of such techniques, see Hobbs S. or Murry L.E. (1992) in McGraw Hill Yearbook of Science and Technology, McGraw Hill, New York, N.Y., pp 191-196; or Weissbach and Weissbach (1988) *Methods for Plant Molecular Biology*, Academic Press, New York, N.Y., pp 421-463.

Variant product may also be expressed in an insect system. In one such system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. The variant product coding sequence may be cloned into a nonessential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of variant coding sequence will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein coat. The recombinant viruses are then used to infect *S. frugiperda* cells or *Trichoplusia* larvae in which variant protein is expressed (Smith *et al., J. Virol.* 46:584, (1983); Engelhard, E.K. *et al., Proc. Nat. Acad. Sci.* **91**:3224-7, (1994)).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, a variant product coding sequence may be ligated into an adenovirus

transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a nonessential E1 or E3 region of the viral genome will result in a viable virus capable of expressing variant protein in infected host cells (Logan and Shenk, *Proc. Natl. Acad. Sci.* **81**:3655-59, (1984). In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Specific initiation signals may also be required for efficient translation of a variant product coding sequence. These signals include the ATG initiation codon and adjacent sequences. In cases where variant product coding sequence, its initiation codon and upstream sequences are inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only coding sequence, or a portion thereof, is inserted, exogenous transcriptional control signals including the ATG initiation codon must be provided. Furthermore, the initiation codon must be in the correct reading frame to ensure transcription of the entire insert. Exogenous transcriptional elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate to the cell system in use (Scharf, D. *et al.,* (1994) *Results Probl. Cell Differ.,* **20**:125-62, (1994); Bittner et al., *Methods in Enzymol* **153**:516-544, (1987)).

In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (Davis, L., Dibner, M., and Battey, I. (1986) Basic Methods in Molecular Biology). Cell-free translation systems can also be employed to produce polypeptides using RNAs derived from the DNA constructs of the present invention.

A host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the protein include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation. Post-translational processing which cleaves a *"pre-pro"* form of the protein may also be important for correct insertion, folding and/or function. Different host cells such as CHO, HeLa, MDCK, 293, WI38, etc. have specific cellular machinery and characteristic mechanisms for such post-translational activities and may be chosen to ensure the correct modification and processing of the introduced, foreign protein.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express variant product may be transformed using expression vectors which contain viral origins of replication or endogenous expression elements and a selectable marker gene. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clumps of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler M., *et al.*, *Cell* **11**:223-32, (1977)) and adenine phosphoribosyltransferase (Lowy I., *et al.*, *Cell* **22**:817-23, (1980)) genes which can be employed in *tk-* or *aprt-* cells, respectively. Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, *dhfr* which confers resistance to methotrexate (Wigler M., *et al., Proc. Natl. Acad. Sci.* 77:3567-70, (1980)); *npt*, which confers resistance to the aminoglycosides neomycin and G-418 (Colbere-Garapin, F. *et al., J. Mol. Biol.*, **150**:1-14, (1981)) and *als* or *pat*, which confer resistance to chlorsulfuron and

phosphinotricin acetyltransferase, respectively (Murry, *supra*). Additional selectable genes have been described, for example, *trpB*, which allows cells to utilize indole in place of tryptophan, or *hisD*, which allows cells to utilize histinol in place of histidine (Hartman S.C. and R.C. Mulligan, *Proc. Natl. Acad. Sci.* **85**:8047-51, (1988)). The use of visible markers has gained popularity with such markers as anthocyanins, beta-glucuronidase and its substrate, GUS, and luciferase and its substrates, luciferin and ATP, being widely used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes, C.A. *et. al.*, *Methods Mol. Biol.*, 55:121-131, (1995)).

Host cells transformed with a nucleotide sequence encoding variant product may be cultured under conditions suitable for the expression and recovery of the encoded protein from cell culture. The product produced by a recombinant cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing nucleic acid sequences encoding variant product can be designed with signal sequences which direct secretion of variant product through a prokaryotic or eukaryotic cell membrane.

The variant product may also be expressed as a recombinant protein with one or more additional polypeptide domains added to facilitate protein purification. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp, Seattle, Wash.). The inclusion of a protease-cleavable polypeptide linker sequence between the purification domain and variant product is useful to facilitate purification. One such expression vector provides for expression of a fusion protein compromising a variant polypeptide fused to a polyhistidine region separated by an enterokinase cleavage site. The histidine residues facilitate purification on IMIAC

(immobilized metal ion affinity chromatography, as described in Porath, *et al.*, *Protein Expression and Purification,* **3**:263-281, (1992)) while the enterokinase cleavage site provides a means for isolating variant polypeptide from the fusion protein. *pGEX* vectors (Promega, Madison, Wis.) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to ligand-agarose beads (e.g., glutathione-agarose in the case of GST-fusions) followed by elution in the presence of free ligand.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification. Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, or other methods, which are well know to those skilled in the art.

The variant products can be recovered and purified from recombinant cell cultures by any of a number of methods well known in the art, including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography, and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

C. Diagnostic applications utilizing nucleic acid sequences

The nucleic acid sequences of the present invention may be used for a variety of diagnostic purposes. The nucleic acid sequences may be used to detect

and quantitate expression of the variant in patient's cells, e.g. biopsied tissues, by detecting the presence of mRNA coding for variant product. Alternatively, the assay may be used to detect soluble variant in the serum or blood. This assay typically involves obtaining total mRNA from the tissue or serum and contacting the mRNA with a nucleic acid probe. The probe is a nucleic acid molecule of at least 20 nucleotides, preferably 20-30 nucleotides, capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding variant product under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of variant. This assay can be used to distinguish between absence, presence, and excess expression of variant product and to monitor levels of variant expression during therapeutic intervention. In addition, the assay may be used to compare the levels of the variant of the invention to the levels of the original sequence from which it has been varied or to levels of other variants, which comparison may have some physiological meaning.

The invention also contemplates the use of the nucleic acid sequences as a diagnostic for diseases resulting from inherited defective variant sequences, or diseases in which the ratio of the amount of the original sequence from which the variant was varied to the novel variants of the invention is altered. These sequences can be detected by comparing the sequences of the defective (i.e., mutant) variant coding region with that of a normal coding region. Association of the sequence coding for mutant variant product with abnormal variant product activity may be verified. In addition, sequences encoding mutant variant products can be inserted into a suitable vector for expression in a functional assay system (e.g., colorimetric assay, complementation experiments in a variant protein deficient strain of HEK293 cells) as yet another means to verify or identify mutations. Once mutant genes have been identified, one can then screen populations of interest for carriers of the mutant gene.

Individuals carrying mutations in the nucleic acid sequence of the present invention may be detected at the DNA level by a variety of techniques. Nucleic

acids used for diagnosis may be obtained from a patient's cells, including but not limited to such as from blood, urine, saliva, placenta, tissue biopsy and autopsy material. Genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR (Saiki, *et al.*, *Nature* **324**:163-166, (1986)) prior to analysis. RNA or cDNA may also be used for the same purpose. As an example, PCR primers complementary to the nucleic acid of the present invention can be used to identify and analyze mutations in the gene of the present invention. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype.

Point mutations can be identified by hybridizing amplified DNA to radiolabeled RNA of the invention or alternatively, radiolabeled antisense DNA sequences of the invention. Sequence changes at specific locations may also be revealed by nuclease protection assays, such RNase and S1 protection or the chemical cleavage method (e.g. Cotton, *et alProc. Natl. Acad. Sci.* USA, **85**:4397-4401, (1985)), or by differences in melting temperatures. *"Molecular beacons"* (Kostrikis L.G. *et al.*, Science **279**:1228-1229, (1998)), hairpin-shaped, single-stranded synthetic oligo- nucleotides containing probe sequences which are complementary to the nucleic acid of the present invention, may also be used to detect point mutations or other sequence changes as well as monitor expression levels of variant product. Such diagnostics would be particularly useful for prenatal testing.

Another method for detecting mutations uses two DNA probes which are designed to hybridize to adjacent regions of a target, with abutting bases, where the region of known or suspected mutation(s) is at or near the abutting bases. The two probes may be joined at the abutting bases, e.g., in the presence of a ligase enzyme, but only if both probes are correctly base paired in the region of probe junction. The presence or absence of mutations is then detectable by the presence or absence of ligated probe.

Also suitable for detecting mutations in the variant product coding sequence are oligonucleotide array methods based on sequencing by

hybridization (SBH), as described, for example, in U.S. Patent No. 5,547,839. In a typical method, the DNA target analyte is hybridized with an array of oligonucleotides formed on a microchip. The sequence of the target can then be "read" from the pattern of target binding to the array.

D. Gene mapping utilizing nucleic acid sequences

The nucleic acid sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 20-30 bp) from the variant cDNA. Computer analysis of the 3' untranslated region is used to rapidly select primers that do not span more than one exon in the genomic DNA, which would complicate the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the primer will yield an amplified fragment.

PCR mapping of somatic cell hybrids or using instead radiation hybrids are rapid procedures for assigning a particular DNA to a particular chromosome. Using the present invention with the same oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes or pools of large genomic clones in an analogous manner. Other mapping strategies that can similarly be used to map to its chromosome include *in situ* hybridization, prescreening with labeled flow-sorted chromosomes and preselection by hybridization to construct chromosome specific-cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with cDNA as short as 50 or 60 bases. For a review of this technique, see Verma *et al., Human Chromosomes: a Manual of Basic Techniques*, (1988) Pergamon Press, New York.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in the OMIM database (Center for Medical Genetics, Johns Hopkins University, Baltimore, MD and National Center for Biotechnology Information, National Library of Medicine, Bethesda, MD). The OMIM gene map presents the cytogenetic map location of disease genes and other expressed genes. The OMIM database provides information on diseases associated with the chromosomal location. Such associations include the results of linkage analysis mapped to this interval, and the correlation of translocations and other chromosomal aberrations in this area with the advent of polygenic diseases, such as cancer, in general and prostate cancer in particular.

E. Therapeutic applications of nucleic acid sequences

Nucleic acid sequences of the invention may also be used for therapeutic purposes. Turning first to the second aspect of the invention (i.e. inhibition of expression of variant), expression of variant product may be modulated through antisense technology, which controls gene expression through hybridization of complementary nucleic acid sequences, i.e. antisense DNA or RNA, to the control, 5' or regulatory regions of the gene encoding variant product. For example, the 5' coding portion of the nucleic acid sequence sequence which codes for the product of the present invention is used to design an antisense oligonucleotide of from about 10 to 40 base pairs in length. Oligonucleotides derived from the transcription start site, e.g. between positions -10 and +10 from the start site, are preferred. An antisense DNA oligonucleotide is designed to be

complementary to a region of the nucleic acid sequence involved in transcription (Lee *et al., Nucl. Acids, Res.*, **6**:3073, (1979); Cooney et al., Science **241**:456, (1988); and Dervan *et al., Science* **251**:1360, (1991)), thereby preventing transcription and the production of the variant products. An antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into the variant products (Okano *J. Neurochem.* **56**:560, (1991)). The antisense constructs can be delivered to cells by procedures known in the art such that the antisense RNA or DNA may be expressed *in vivo*. The antisense may be antisense mRNA or DNA sequence capable of coding such antisense mRNA. The antisense mRNA or the DNA coding thereof can be complementary to the full sequence of nucleic acid sequences coding for the variant protein or to a fragment of such a sequence which is sufficient to inhibit production of a protein product.

Turning now to the first aspect of the invention, i.e. expression of variant, expression of variant product may be increased by providing coding sequences for coding for said product under the control of suitable control elements ending its expression in the desired host.

The nucleic acid sequences of the invention may be employed in combination with a suitable pharmaceutical carrier. Such compositions comprise a therapeutically effective amount of the compound, and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The formulation should suit the mode of administration.

The products of the invention as well as any activators and deactivators compounds (see below) which are polypeptides, may also be employed in accordance with the present invention by expression of such polypeptides *in vivo*, which is often referred to as *"gene therapy."* Cells from a patient may be engineered with a nucleic acid sequence (DNA or RNA) encoding a polypeptide *ex vivo*, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, cells

may be engineered by procedures known in the art by use of a retroviral particle containing RNA encoding a polypeptide of the present invention.

Similarly, cells may be engineered in vivo for expression of a polypeptide *in vivo* by procedures known in the art. As known in the art, a producer cell for producing a retroviral particle containing RNA encoding the polypeptide of the present invention may be administered to a patient for engineering cells *in vivo* and expression of the polypeptide *in vivo*. These and other methods for administering a product of the present invention by such method should be apparent to those skilled in the art from the teachings of the present invention. For example, the expression vehicle for engineering cells may be other than a retrovirus, for example, an adenovirus which may be used to engineer cells *in vivo* after combination with a suitable delivery vehicle.

Retroviruses from which the retroviral plasmid vectors mentioned above may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, adenovirus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the *PE501*, *PA317*, *psi-2*, *psi-AM*, *PA12*, *T19-14X*, *VT-19-17-H2*, *psi-CRE*, *psi-CRIP*, *GP+E-86*, *GP+envAm12*, and *DAN* cell lines as described in Miller (*Human Gene Therapy*, Vol. **1**, pg. 5-14, (1990)). The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and $CaPO_4$ precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

The producer cell line generates infectious retroviral vector particles which include the nucleic acid sequence(s) encoding the polypeptides. Such

retroviral vector particles then may be employed, to transduce eukaryotic cells, either in vitro or in vivo. The transduced eukaryotic cells will express the nucleic acid sequence(s) encoding the polypeptide. Eukaryotic cells which may be transduced include, but are not limited to, embryonic stem cells, embryonic carcinoma cells, as well as hematopoietic stem cells, hepatocytes, fibroblasts, myoblasts, keratinocytes, endothelial cells, and bronchial epithelial cells.

The genes introduced into cells may be placed under the control of inducible promoters, such as the radiation-inducible Egr-1 promoter, (Maceri, H.J., *et al., Cancer Res.*, **56**(19):4311 (1996)), to stimulate variant production or antisense inhibition in response to radiation, eg., radiation therapy for treating tumors.

Example III. Variant product

The substantially purified variant product of the invention has been defined above as the product coded from the nucleic acid sequence of the invention. Preferably the amino acid sequence is an amino acid sequence having at least 90% identity to any one of the sequences coded by the nucleic acid sequence of SEQ ID NO:1 to SEQ ID NO:48611 provided that the amino acid sequence is not identical to that of the original sequence from which it has been varied. The protein or polypeptide may be in mature and/or modified form, also as defined above. Also contemplated are protein fragments having at least 10 contiguous amino acid residues, preferably at least 10-20 residues, derived from the variant product, as well as homologues as explained above.

The sequence variations are preferably those that are considered conserved substitutions, as defined above. Thus, for example, a protein with a sequence having at least 90% sequence identity with any of the products coded by SEQ ID NO: 1 to SEQ ID NO:48611, preferably by utilizing conserved substitutions as defined above is also part of the invention, and provided that it is not identical to the original peptide from which it has been varied. The variant product may be (i) one in which one or more of the amino acid residues in a

sequence listed above are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue), or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the variant product is fused with another compound, such as a compound to increase the half-life of the protein (for example, polyethylene glycol (PEG)), or a moiety which serves as targeting means to direct the protein to its target tissue or target cell population (such as an antibody), or (iv) one in which additional amino acids are fused to the variant product. Such fragments, variants and derivatives are deemed to be within the scope of those skilled in the art from the teachings herein.

A. Preparation of variant product

Recombinant methods for producing and isolating the variant product, and fragments of the protein are described above.

In addition to recombinant production, fragments and portions of variant product may be produced by direct peptide synthesis using solid-phase techniques (cf. Stewart *et al.*, (1969) Solid-Phase Peptide Synthesis, WH Freeman Co, San Francisco; Merrifield J., *J. Am. Chem. Soc.,* **85**:2149-2154, (1963)). In vitro peptide synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer, Foster City, Calif.) in accordance with the instructions provided by the manufacturer. Fragments of variant product may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

B. Therapeutic uses and compositions utilizing the variant product

The variant product of the invention is generally useful in treating diseases and disorders which are characterized by a lower than normal level of variant expression, and or diseases which can be cured or ameliorated by raising the level of the variant product, even if the level is normal.

Variant products or fragments may be administered by any of a number of routes and methods designed to provide a consistent and predictable concentration of compound at the target organ or tissue. The product-containing compositions may be administered alone or in combination with other agents, such as stabilizing compounds, and/or in combination with other pharmaceutical agents such as drugs or hormones.

Variant product-containing compositions may be administered by a number of routes including, but not limited to oral, intravenous, intramuscular, transdermal, subcutaneous, topical, sublingual, or rectal means as well as by nasal application. variant product-containing compositions may also be administered via liposomes. Such administration routes and appropriate formulations are generally known to those of skill in the art.

The product can be given via intravenous or intraperitoneal injection. Similarly, the product may be injected to other localized regions of the body. The product may also be administered via nasal insufflation. Enteral administration is also possible. For such administration, the product should be formulated into an appropriate capsule or elixir for oral administration, or into a suppository for rectal administration.

The foregoing exemplary administration modes will likely require that the product be formulated into an appropriate carrier, including ointments, gels, suppositories. Appropriate formulations are well known to persons skilled in the art.

Dosage of the product will vary, depending upon the potency and therapeutic index of the particular polypeptide selected.

A therapeutic composition for use in the treatment method can include the product in a sterile injectable solution, the polypeptide in an oral delivery vehicle, the product in an aerosol suitable for nasal administration, or the product in a nebulized form, all prepared according to well known methods. Such compositions comprise a therapeutically effective amount of the compound, and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof.

Example IV. Screening methods for activators and deactivators (inhibitors)

The present invention also includes an assay for identifying molecules, such as synthetic drugs, antibodies, peptides, or other molecules, which have a modulating effect on the activity of the variant product, e.g. activators or deactivators of the variant product of the present invention. Such an assay comprises the steps of providing an variant product encoded by the nucleic acid sequences of the present invention, contacting the variant protein with one or more candidate molecules to determine the candidate molecules modulating effect on the activity of the variant product, and selecting from the molecules a candidate's molecule capable of modulating variant product physiological activity.

The variant product, its catalytic or immunogenic fragments or oligopeptides thereof, can be used for screening therapeutic compounds in any of a variety of drug screening techniques. The fragment employed in such a test may be free in solution, affixed to a solid support, borne on a cell membrane or located intracellularly. The formation of binding complexes, between variant product and the agent being tested, may be measured. Alternatively, the activator or deactivator may work by serving as agonist or antagonist, respectively, of the

variant receptor, binding entity or target site, and their effect may be determined in connection with any of the above.

Another technique for drug screening which may be used provides for high throughput screening of compounds having suitable binding affinity to the variant product is described in detail by Geysen in PCT Application WO 84/03564, published on Sep. 13, 1984. In summary, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with the full variant product or with fragments of variant product and washed. Bound variant product is then detected by methods well known in the art. Substantially purified variant product can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

Antibodies to the variant product, as described in Example VI below, may also be used in screening assays according to methods well known in the art. For example, a *"sandwich"* assay may be performed, in which an anti-variant antibody is affixed to a solid surface such as a microtiter plate and variant product is added. Such an assay can be used to capture compounds which bind to the variant product. Alternatively, such an assay may be used to measure the ability of compounds to influence with the binding of variant product to the variant receptor, and then select those compounds which effect the binding.

Example VI. Anti-variant antibodies/distinguishing antibodies

A. Synthesis

In still another aspect of the invention, the purified variant product is used to produce anti-variant antibodies which have diagnostic and therapeutic uses related to the activity, distribution, and expression of the variant product. As indicated above, the antibodies may also be directed solely to amino acid sequences present in the variant but not present in the original sequence, or to

sequences present only in the original sequence but not in the variant (distinguishing antibodies).

Antibodies to the variant product or to the distinguishing sequence present only in the variant or only in the original sequence (the latter termed *"distinguishing antibodies"*) may be generated by methods well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, humanized, single chain, Fab fragments and fragments produced by an Fab expression library. Antibodies, i.e., those which inhibit dimer formation, are especially preferred for therapeutic use.

A fragment of the variant product for antibody induction does not require biological activity but have to feature immunological activity; however, the protein fragment or oligopeptide must be antigenic. Peptides used to induce specific antibodies may have an amino acid sequence consisting of at least five amino acids, preferably at least 10 amino acids of any sequences coded by the nucleic acid sequence of SEQ ID NO:1 to SEQ ID NO:48611 or in distinguishing sequences present only in the variant or only in the original sequence as explained above. Preferably they should mimic a portion of the amino acid sequence of the natural protein and may contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of variant protein amino acids may be fused with those of another protein such as keyhole limpet hemocyanin and antibody produced against the chimeric molecule. Procedures well known in the art can be used for the production of antibodies to variant product.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, etc may be immunized by injection with variant product or any portion, fragment or oligopeptide which retains immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include but are not limited to Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole

limpet hemocyanin, and dinitrophenol. BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are potentially useful human adjuvants.

Monoclonal antibodies to variant protein may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include but are not limited to the hybridoma technique originally described by Koehler and Milstein (*Nature* **256**:495-497, (1975)), the human B-cell hybridoma technique (Kosbor *et al., Immunol. Today* **4**:72, (1983); Cote *et al., Proc. Natl. Acad. Sci.* **80**:2026-2030, (1983)) and the EBV-hybridoma technique (Cole, *et al., Mol. Cell Biol.* 62:109-120, (1984)).

Techniques developed for the production of *"chimeric antibodies",* the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity can also be used (Morrison *et al., Proc. Natl. Acad. Sci.* **81**:6851-6855, (1984); Neuberger *et al., Nature* **312**:604-608, (1984); Takeda *et al., Nature* **314**:452-454, (1985)). Alternatively, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,946,778) can be adapted to produce single-chain antibodies specific for the variant protein.

Antibodies may also be produced by inducing *in vivo* production in the lymphocyte population or by screening recombinant immunoglobulin libraries or panels of highly specific binding reagents as disclosed in Orlandi *et al.* (*Proc. Natl. Acad. Sci.* **86**:3833-3837, 1989)), and Winter G and Milstein C., (*Nature* **349**:293-299, (1991)).

Antibody fragments which contain specific binding sites for variant protein may also be generated. For example, such fragments include, but are not limited to, the $F(ab')_2$ fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of

monoclonal Fab fragments with the desired specificity (Huse W.D. *et al., Science* 256:1275-1281, (1989)).

B. Diagnostic applications of antibodies

A variety of protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the formation of complexes between the variant product and its specific antibody and the measurement of complex formation. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two noninterfering epitopes on a specific variant product is preferred, but a competitive binding assay may also be employed. These assays are described in Maddox D.E., *et al.,* (*J. Exp. Med.* **158**:1211, (1983)).

Antibodies which specifically bind variant product or distinguishing antibodies which bind to sequences which distinguish the variant from the original sequence (as explained above) are useful for the diagnosis of conditions or diseases characterized by expression of the novel variant of the invention (where normally it is not expressed) by over or under expression of variant as well as for detection of diseases in which the proportion between the amount of the variants of the invention and the original sequence from which it varied is altered. Alternatively, such antibodies may be used in assays to monitor patients being treated with variant product, its activators, or its deactivators. Diagnostic assays for variant protein include methods utilizing the antibody and a label to detect variant product in human body fluids or extracts of cells or tissues. The products and antibodies of the present invention may be used with or without modification. Frequently, the proteins and antibodies will be labeled by joining them, either covalently or noncovalently, with a reporter molecule. A wide variety of reporter molecules are known in the art.

A variety of protocols for measuring the variant product, using either polyclonal or monoclonal antibodies specific for the respective protein are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescent activated cell sorting (FACS). As noted above, a two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on variant product is preferred, but a competitive binding assay may be employed. These assays are described, among other places, in Maddox, *et al. (supra).* Such protocols provide a basis for diagnosing altered or abnormal levels of variant product expression. Normal or standard values for variant product expression are established by combining body fluids or cell extracts taken from normal subjects, preferably human, with antibody to variant product under conditions suitable for complex formation which are well known in the art. The amount of standard complex formation may be quantified by various methods, preferably by photometric methods. Then, standard values obtained from normal samples may be compared with values obtained from samples from subjects potentially affected by disease. Deviation between standard and subject values establishes the presence of disease state.

The antibody assays are useful to determine the level of variant product present in a body fluid sample, in order to determine whether it is being expressed at all, whether it is being overexpressed or underexpressed in the tissue, or as an indication of how variant levels of variable products are responding to drug treatment.

C. Therapeutic uses of antibodies

In addition to their diagnostic use the antibodies may have a therapeutical utility in blocking or decreasing the activity of the variant product in pathological conditions where beneficial effect can be achieved by such a decrease. Again, distinguishing antibodies may be used to neutralize differentially either the variant or the original sequence as the case may be.

The antibody employed is preferably a humanized monoclonal antibody, or a human Mab produced by known globulin-gene library methods. The antibody is administered typically as a sterile solution by IV injection, although other parenteral routes may be suitable. Typically, the antibody is administered in an amount between about 1-15 mg/kg body weight of the subject. Treatment is continued, e.g., with dosing every 1-7 days, until a therapeutic improvement is seen.

Although the invention has been described with reference to specific methods and embodiments, it is appreciated that various modifications and changes may be made without departing from the invention.